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Particle-associated bacterial dynamics in a tropical tidal plain (Zuari estuary, India)

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ABSTRACT: The dynamics of particle-associated bacteria (PAB) in a tropical estuary, Zuari, in Goa on the west coast of India, was studied for a year from September 1997 to October 1998. Bacterial abundance, productivity and enzymatic activities were measured at a fixed station in the estuary. The study period covered pre-monsoon (February to May), southwest monsoon (June to September) and post-monsoon (October to January) seasons. Particulate organic carbon ranged from 0.5 to 17.5 mg C l^{-1}. Particles of size range >3 to <220 µm were the most abundant, were rich in organic carbon (~60%), and formed 55% of the suspended load (ave. ~0.1 g l^{-1}) in the estuary. PAB ranged from 8.8×10^7 to 3.9×10^{10} l^{-1} and the waters close to the sediment had a higher abundance than the surface waters. PAB varied seasonally and accounted for 20 to 80% of the total bacterial population. The mean rate of production as measured by ^{3}H-thymidine incorporation was 70 µg C l^{-1} d^{-1} in the surface and 35 µg C l^{-1} d^{-1} in the bottom waters. Monthly production varied by 2 orders and was not coupled to abundance. However, high production during the monsoon was fuelled by allochthonous input of organic matter. No linear relationship was observed between biological parameters and other measured environmental parameters except for temperature, which showed a linear relationship with PAB and particle numbers. The bacterial carbon demand (BCD) at the station was higher than the primary production (PP) and was met by allochthonous input. Our study demonstrates that the PAB dynamics were determined by the availability of substrates and resuspension of sedimented materials.

KEY WORDS: Particle-associated bacteria · Biomass · Productivity · Tropical · Estuary

INTRODUCTION

Estuarine environments exhibit a wide variation in physical and chemical factors, both in space and time. One may expect differences in biota and the biotic dynamics in relation to morphology, catchments, allochthonous inputs and other chemical and physiological factors. Irrespective of the estuary, particles are known to be a major component of the system (Eisma 1993, Crump et al. 1999). They may be retained either in the estuary or transported to the adjoining coastal waters depending upon the magnitude of the riverine flow and the tide. Particles are formed of suspended organic and inorganic matter produced by biologically enhanced physical processes (Kiørboe et al. 1990). These fragile microscopic particles are composed of fecal pellets, decaying macrophytes and phytoplankton, mineral and detrital particles (Alldredge & Silver 1988, Velmirov 1991). Bacteria that colonize such aggregates in estuaries form a significant fraction of the total bacterial population in contrast to the larger size aggregates of the open oceans (Bell & Albright 1981, Iriberri et al. 1987, Zimmermann & Kausch 1996, Zimmermann 1997, Grossart & Simon 1998). It has been generally observed that the density of bacteria on particles is higher than that of free-living bacteria in the water (Caron et al. 1986, Caron 1987, Müller-Niklas et al. 1994, Turley & Mackie 1994). Adsorbed material, availability of potential substrates, organic components of particles, and increased flux of nutrients are some of the reasons why particles form a favorable microenvironment for bacterial colonization (Fletcher 1991, Kirchman 1993). Particle-associated bacteria (PAB) also seem to contribute to the break-

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down of macroaggregates by mechanical disruption and direct consumption of the particulate material (Lochte 1991, Zimmermann & Kausch 1996). Thus the estuary forms an important site for bacterial degradation of terrestrial and riverine organic matter associated with particles (Lee & Wakeham 1988).

PAB are now recognized as an important component of the food web in the estuarine system (Crump et al. 1999) because they tend to be retained in the estuary either by sinking or re-suspension (Crump et al. 1998). In temperate estuaries, like that of the Columbia River in the northwestern USA, particle-associated/attached bacteria have been shown to account for 90% of the total bacterial production (Crump et al. 1998), and in the Elbe Estuary (Germany) they are 4×10^3 times more concentrated than the free-living bacteria (Zimmermann 1997). Thus, particles are not only a site for decomposition but also form an important component of the food web. The available studies on estuaries, however, do not allow a generalization of the parameters controlling bacterial dynamics, probably due to the high environmental variability of these ecosystems. For instance in the Gambia River, West Africa, the dominance of attached bacteria was related to high seston loads (Healey et al. 1988), while in turbid St. Lawrence Estuary, Canada, attached bacteria were found to play a minor role (Painchaud & Therriault 1989). The absolute amount of bacteria and relative contribution by particle-associated bacteria are expected to vary among estuaries because of differences in organic matter quality and quantity. Thus a combination of intrinsic and extrinsic controlling factors may account for magnifying differences among the estuaries.

Very little work on PAB has been carried out in tropical estuaries except in a tidal creek of the Indus delta, Pakistan (Bano et al. 1997), in the Gambia River (Healey et al. 1988), and in the Ivory Coast (Ducklow & Shiah 1993). No study has been made on an annual basis to understand the seasonal changes in PAB in a tropical estuary. At the study station in the Zuari estuary (Goa, India), seasonality is brought about by the southwest (SW) monsoon, which is accompanied by high influx of fresh water. There is a comparatively low run-off season during November to May as compared to the heavy run off period from June to October (Shetye et al. 1995). The quality, timing and characteristic of allochthonous loading may affect the system. Hence, a study on bacterial activities on particles is of immediate relevance. We do not know whether (1) this estuary forms an important energetic link between riverine and marine fronts like the temperate estuaries, or (2) wind-driven monsoons influence the particles and their associated bacteria.

The objective of our study was to describe how PAB in the Zuari estuary respond to physico-chemical and biological changes during an annual cycle. In this paper we (1) quantify the abundance of bacteria on particles in the water column and their contribution to the total microbial activity, and (2) bring out the importance of attached bacteria to the status of the estuary.

MATERIALS AND METHODS

Study site and sampling. The Zuari estuary located in Goa on the west coast of India is the terminus for a >50 km long river that empties into the Arabian Sea. It is fringed by mangroves and referred to as a tidal plain estuary or a partially mixed estuary. The bottom topography is flat, with very few trenches as deep as 10 m. The cross-sectional area of the estuary decreases exponentially. This decrease is more rapid in the first 20 km from the 5 km wide mouth. The amplitudes during spring tide and neap tide were 2.3 and 1 m respectively (Shetye & Murty 1987).

The SW monsoon from June to September is characterized by heavy rainfall (~2500 mm). The post-monsoon season, October to January, is dry and relatively cool, whereas the pre-monsoon from February to May is hot and humid. The characteristics of the Zuari estuary differ markedly between a low run-off season between November and May and a heavy run-off period of the southwest monsoon from June to September. From November to May the estuary is vertically mixed and the 2 processes controlling the transport of salt are (1) run-off-induced advective transport out of the estuary, and (2) tidally induced diffusive transport into the estuary. The magnitude of the latter is ca. 20% larger than the former, leading to a salinity rise in the estuary. The intrusion of salt water is seen as far as 65 km upstream in May, and reduced to a minimum of 20 km in June (Qasim & Sen Gupta 1981). With the onset of the SW monsoon, the run-off increases dramatically and the estuary loses ca. 75% of its salt during the first 2 mo. Because the estuary is partially stratified from June to October, gravitational circulation is expected to play a role in addition to tidal diffusion and run off.

The sampling site was at 15° 25.107′ N and 73° 51.472′ E (Fig. 1). The average depth during the sampling months was approximately 4 m. Strong changes in depth were not seen, as the change in mean sea level was only 10 cm. Monthly water samples from the surface and from close to the bottom, i.e. just above the sediment layer, were collected at low tide in the mid reach of the estuary from October 1997 to September 1998. Samples were taken in acid-cleaned poly-

propylene bottles after pre-filtration through 220 µm mesh to remove larger plankton and processed in the laboratory within 2 h of sampling.

Physicochemical parameters. Water temperature, pH and salinity (AUTOSAL [8400 A]) were measured. Suspended particulate matter (SPM) was determined gravimetrically on pre-weighed GF/F (Whatman) filters as described by Krey (1964). After weighing, the filters were ignited at 450°C for 3 h and re-weighed after cooling in order to determine the particulate inorganic matter (PIM). Blank filters were also maintained to determine any weight changes. Particulate organic matter (POM) was calculated by subtracting PIM values from SPM. For the estimation of particulate organic carbon (POC) and particulate organic nitrogen (PON), the pre-filtered (220 µm) water samples were filtered through pre-combusted GF/D (Whatman) filters and dried at 60°C. After the filters were fumed with concentrated HCl for 24 h to remove carbonates (Hedges & Stern 1984), they were analyzed using a Perkin Elmer Elemental CHN analyzer (Model 2400). The C:N ratio was estimated in a CHN analyzer after filtering water samples through GF/D (~3 µm) filters.

Size fractionation. The size distribution of particles in water samples was analyzed using a laser-based particle-size analyzer (Malvern 3600E). Sample dilution, if necessary, was performed with membrane (0.22 µm)-filtered 50% seawater under gentle magnetic stirring for 5 min. For blanks, membrane (0.22 µm)-filtered seawater was used. For the gravimetric method, water samples were sequentially filtered through pre-weighed 3, 2.7, 1.2, 0.7, 0.45 and 0.22 µm pore-size polycarbonate filter (Millipore) under low vacuum (<1 KPa). The filters were then rinsed with 200 ml of membrane-filtered (0.22 µm) distilled water, dried to constant weight at 40°C and weighed. The weights of the different size fractionated particles were expressed as percent of total particle weight. The total number of particles present in the water sample was counted using a Coulter counter (TAIL Model) and the numbers were expressed volumetrically.

Primary production. Primary production (PP) was measured by the $NaH^{14}CO_3$ assimilation method (Lohrenz et al. 1992). Light and dark acid-washed bottles (125 ml capacity) were filled with waters from the surface and close to the sediment. Samples were inoculated with $NaH^{14}CO_3$ (final activity: 5 µCi ml^{-1}, Bhabha Atomic Research Centre [BARC], Bombay) and deck-incubated. Neutral-density screens were employed. On retrieval, the water samples were filtered immediately through a 0.45 µm filter (Millipore, GS type) under diffused light and low pumping pressure (<13 KPa). Radiolabelled dissolved inorganic carbon was removed by exposing the filter papers to fumes of concentrated HCl for 1 min. The filters were then placed in scintillation vials and 5 ml of scintillation cocktail in dioxane (Spectrochem) were added. Radioactivity was measured in a liquid scintillation counter (LKB Wallac 1209). The estimated daily production rates were expressed as µg C m^{-2} d^{-1}.

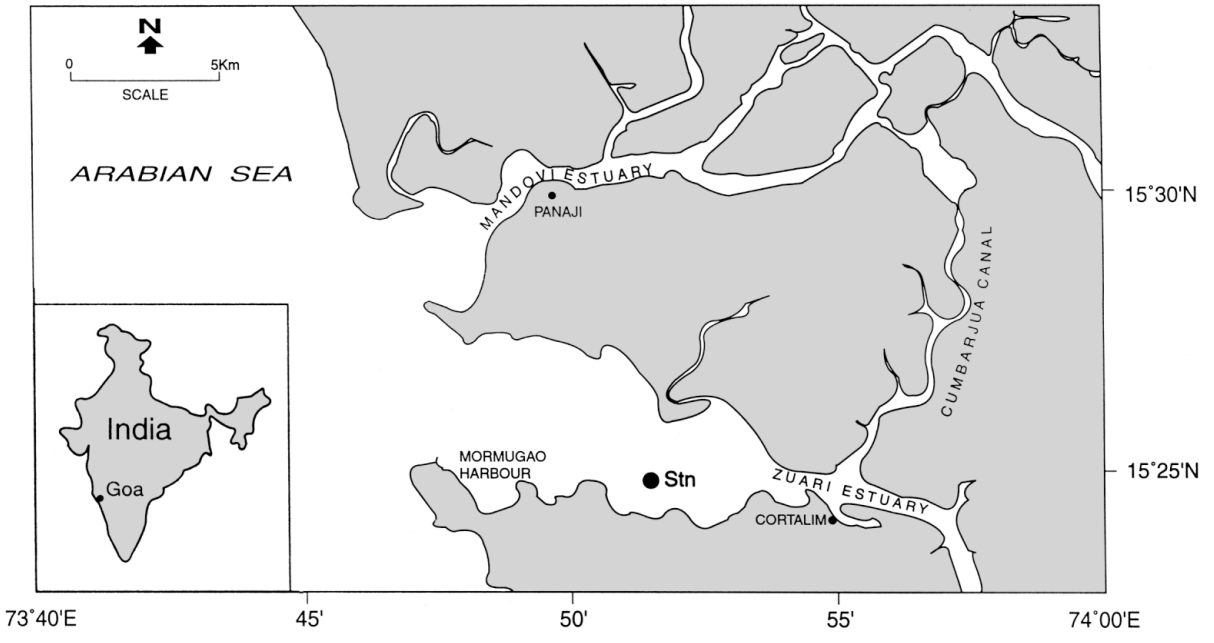

Fig. 1. Map showing the sampling site in Goa on the west coast of India

Bacterial abundance and production. Sub-samples for bacterial abundance and ^{3}H-thymidine incorporation were taken from the whole water samples and from a 3.0 µm pore size pre-filtered (polycarbonate filter) water sample. The abundance and production of PAB were calculated as differences in the parameter between whole water samples and the <3 µm filtrates. To minimize cell damage and the possibility of forcing some large cells through the filter pores, fractionation was done at very low vacuum (<1 kPa) and only a small filtration volume (<100 ml) was used for each filter.

Bacterial numbers were estimated by the acridine orange direct count (AODC) method (Hobbie et al. 1977). Samples were preserved immediately with formaldehyde (2% final concentration). Known amounts of samples were filtered through 0.22 µm black polycarbonate membrane filters (Nuclepore), stained with acridine orange (0.01%) and enumerated using an epifluorescence microscope (BH-Olympus). Counting was done in triplicate with 20 fields counted for each sample, and bacterial numbers were expressed as number per litre.

Bacterial production (BP) was estimated by measuring the rate of [methyl-^{3}H] thymidine incorporation (Fuhrman & Azam 1980, 1982). Water samples (30 ml) were incubated for 1 h with ^{3}H-thymidine (specific activity = 52 Ci mM^{-1}, BARC) at a final concentration of 10 nM. The reaction was terminated with 2% neutral buffered formalin. The samples were then filtered through 0.22 µm polycarbonate filters (pre-soaked in 5% TCA), extracted with cold 5% TCA and rinsed with ethanol. The dried filters were placed in scintillation vials with 3 ml of dioxane-based scintillation cocktail (Sigma) and radio-assayed using a scintillation counter (Tri-CARB 2500 TR Packard). Thymidine incorporation rates were converted to bacterial carbon production (BCP) by using a thymidine conversion factor of 2.0×10^{18} cells produced per mole of thymidine incorporated (Kirchman et al. 1982, Iriberri et al. 1990), and a carbon conversion factor of 2.0×10^{-14} g C cell^{-1} (Lee & Fuhrman 1987). Cell specific production/turnover rate was calculated by dividing the production rate by the bacterial abundance. Bacterial carbon demand (BCD) was calculated on the basis of bacterial carbon production by assuming a carbon assimilation efficiency of a minimum of 10 and a maximum of 30% (Bano et al. 1997), thus covering values found in most studies. BCD was also calculated based on the BGE values of our waters, which ranged from 10 to 26% (Ram et al. 2003).

Enzymatic profile. The ability of PAB to degrade polymers was studied based on their enzyme profile. Water samples (200 ml) were filtered through a 3.0 µm pore size polycarbonate (Millipore) filter. The filter was then transferred to a flask containing 200 ml of sterile 50% seawater, to which 2.0 ml of Tween 80 was added. The flask was kept on a shaker for 15 min and then sonicated for 10 s in order to reduce hydrophobic interactions (Yoon & Rosson 1990) and to dislodge the bacteria from the particles. The suspension was then serially diluted with sterile 50% seawater. The diluted samples were surface plated on nutrient agar medium containing different substrates (2% w/v) for the enzymatic analysis of chitinase, deoxyribonuclease (Dnase), phosphatase, lipase, amylase and protease. The plates were incubated for 24 h at room temperature (28 ± 2°C). Positive colony forming units (CFU) were enumerated and the activity was expressed as the percentage of total colony forming units in each of the substrate.

RESULTS

Water column characteristics

The variation in temperature was minimal and ranged from 27 to 33°C (Table 1). The surface water salinity generally varied from 31 to 34 except during the SW monsoon period where a drastic decrease to 4 was noted. The SPM in the water column fluctuated from 2.6 to 729 mg l^{-1}. The bottom waters recorded twice the SPM of the surface waters. The distribution of particulate organic matter showed the same trend. Size fractionation clearly showed that particles of the size range >3 to <220 µm (Fig. 2) were dominant throughout most of the study period at the surface and bottom waters. The number varied by 1 order of magnitude with the maximum recorded in the bottom waters during the pre-monsoon (February to May). Particle numbers showed a positive relationship with temperature ($r = 0.71$; $p > 0.01$) and salinity ($r = 0.84$; $p > 0.001$) in the surface waters but not in the bottom waters (Table 2).

Table 1. Characteristics of the water column in the Zuari estuary

Parameter	Surface	Bottom
Temperature (°C)	27–33	27–32
pH	6–8	6–8
Salinity	4–34	21–35
SPM (mg l^{-1})	2.6–333	3.4–729
POM (mg l^{-1})	2.6–252	3.4–546
PP (µg C l^{-1} d^{-1})	4.5–63	1.2–25
Particle number ($\times 10^9$ l^{-1})	0.8–2.8	1.4–66
POC (mg C l^{-1})	0.5–18	0.3–8
C:N ratio	2.5–26	3.8–93

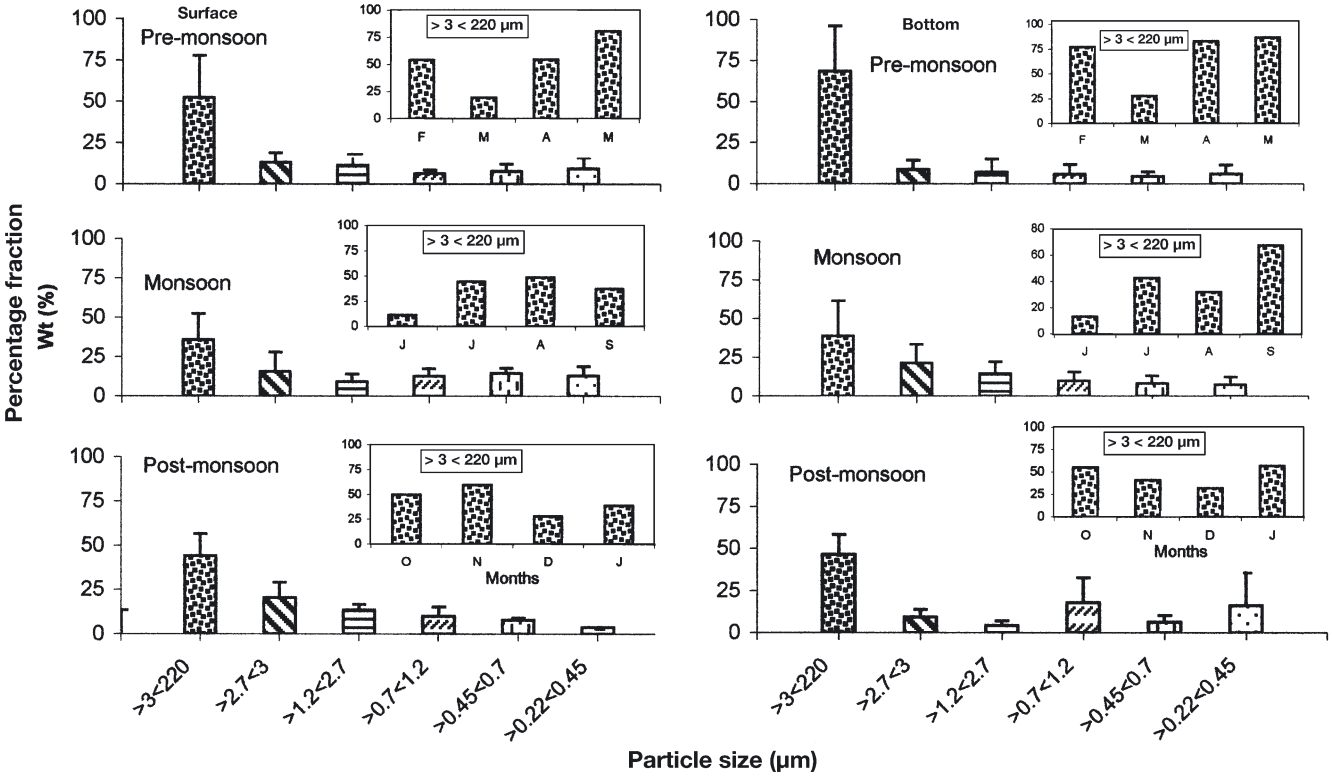

Fig. 2. Seasonal distribution of particle sizes in the water column. Inset shows the monthly dominance of 3–220 µm particles. *y*-axis represents the wt (%) of the respective particle size on the total particles

Bacterial abundance

The mean values of total PAB abundance of the surface waters was 4.06 ± 2.35 × 10^9 l^{-1} and that of the bottom waters was 8.65 ± 10.49 × 10^9 l^{-1}. The average density of PAB was 48% of the total bacterial population, and sometimes reached up to 85% of the total population. As the SW monsoon set in, the bacterial density increased in both the surface and bottom waters, and it dropped towards the end of the season (Fig. 3A). The PAB abundance fluctuated seasonally with a maximum density during the post-monsoon period and a minimum during the pre-monsoon months (Fig. 3B). The annual variation in the PAB density of bottom waters was larger (CV = 140) than the surface waters (CV = 80). The percentage of isolates showing enzyme activity was also high in the bottom waters (Fig. 4). Of the measured environmental variables, temperature showed a negative correlation with PAB abundance. The abundance of PAB was limited by the abundance of total bacteria in the water as a linear relationship was observed throughout the study period for the surface ($r = 0.88$ $p < 0.001$) and bottom waters ($r = 0.98$ $p < 0.001$) (Table 2).

Bacterial production

The rate of production of PAB varied between 11 and 328 µg C l^{-1} d^{-1} at the surface while in the bottom waters it ranged from 1.3 to 109 µg C l^{-1} d^{-1}. The magnitude of

Table 2. Significant relationships between water variables and particle-associated bacteria (PAB) parameters. PP: primary productivity; TBA: total bacterial abundance; TBP: total bacterial production; $^{*}p > 0.05$; $^{**}p > 0.01$; $^{***}p > 0.001$; df = 11

Parameter	Particle number		PAB abundance		PAB production	
	Surface	Bottom	Surface	Bottom	Surface	Bottom
Temperature	0.71**		−0.60*	−0.68*		
Salinity	0.84***					
PP				0.61*		
TBA			0.88***	0.98***		
TBP					0.99***	0.63*

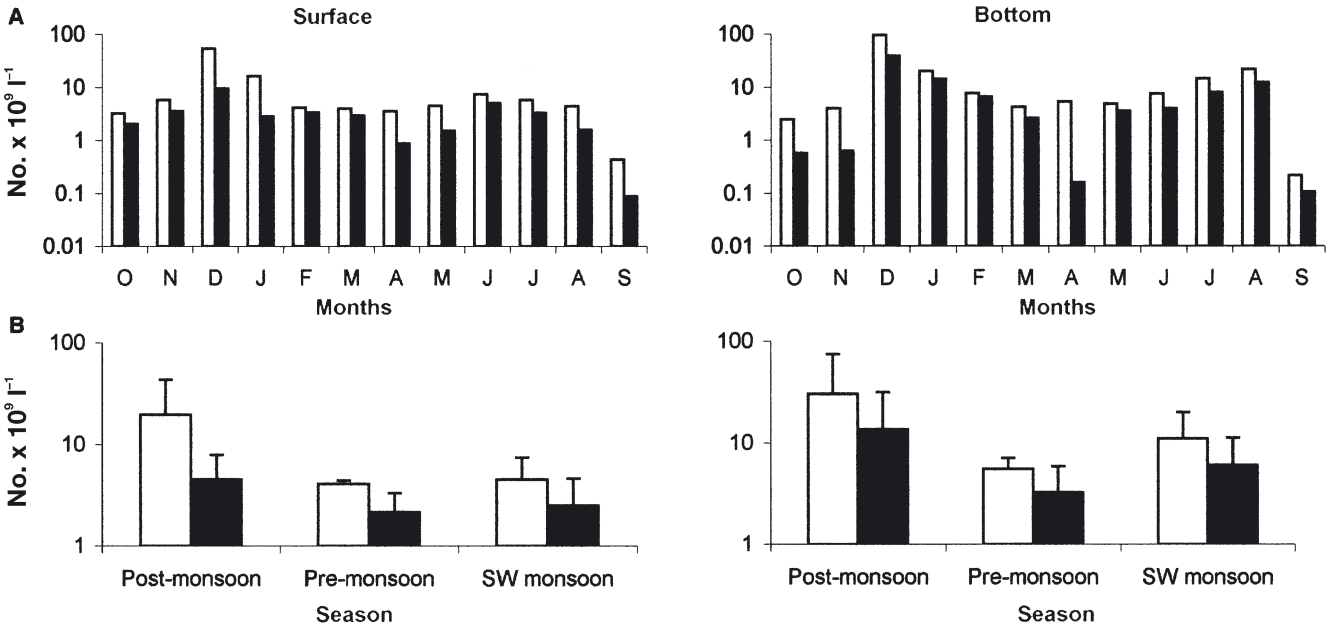

Fig. 3. (A) Monthly and (B) seasonal mean of 4 months variation along with standard deviation, in total and particle associated bacterial (PAB) counts in surface and bottom waters. (□) Total bacteria (■) PAB

variation was 30 times and in the surface waters and 84 times in the bottom waters. PAB contributed ~60% of the total bacterial production (Fig. 5A). Like abundance of PAB, productivity also showed temporal variation with the highest average productivity rate (111 µg C l^{-1} d^{-1}) in the surface waters during the monsoon season. In the bottom waters the average maximum production of 54 µg C l^{-1} d^{-1} was recorded in the post-monsoon season (Fig. 5B). During the SW monsoon, the production was related to the particle numbers ($r = 0.97$ $p < 0.01$), which also showed a significant correlation with salinity ($r = 0.84$ $p > 0.001$); such relationships were restricted to surface waters. The cell-specific production rate was highest during the SW monsoon season in the surface and bottom waters (Fig. 5C). The variation of cell-specific productivity was perhaps governed more by the production rates than by the PAB abundance, as no relationship was observed between PAB abundance and productivity. PAB carbon turnover rates ranged between 0.07 and 2.13 h in the surface waters and 0.2 and 18.5 h in the bottom waters. The variation in the C:N ratio brought about 25% of the variation in PAB carbon turnover rates at the surface waters ($r = 0.50$). Though PAB production accounted for 1.1 to 294% of the PP at the station, it was not related to PP. The BCD ranged from 380 to 990 mg C m^{-2} d^{-1} when a maximum bacterial growth efficiency (BGE) value of 30 was used. The BCD ranged from 438 to 1142 mg C m^{-2} d^{-1} when a BGE value of 26 was used. However, when a BGE of 10 was used values from 1140 to 2970 were obtained (Table 3). Ratios

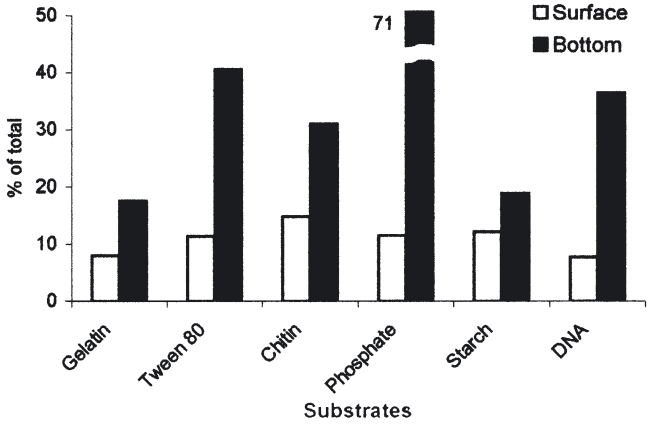

Fig. 4. Percentage of positive PAB on total number of bacteria (CFU)

Table 3. Seasonal variation in particle associated bacterial carbon demand (BCD), based on a bacterial growth efficiency (BGE) of 26 and 10%, and primary production (PP). Values are depth integrated (mg C m^{-2} d^{-1}). 26 and 10% are reported BGE values in the Zuari waters

Season	BCD (BGE of 26/10)	PP	BCD/PP
Pre-monsoon	438/1140	1022	0.4–1.1
SW monsoon	1142/2970	594	1.9–5.0
Post-monsoon	842/2190	717	1.2–3.1

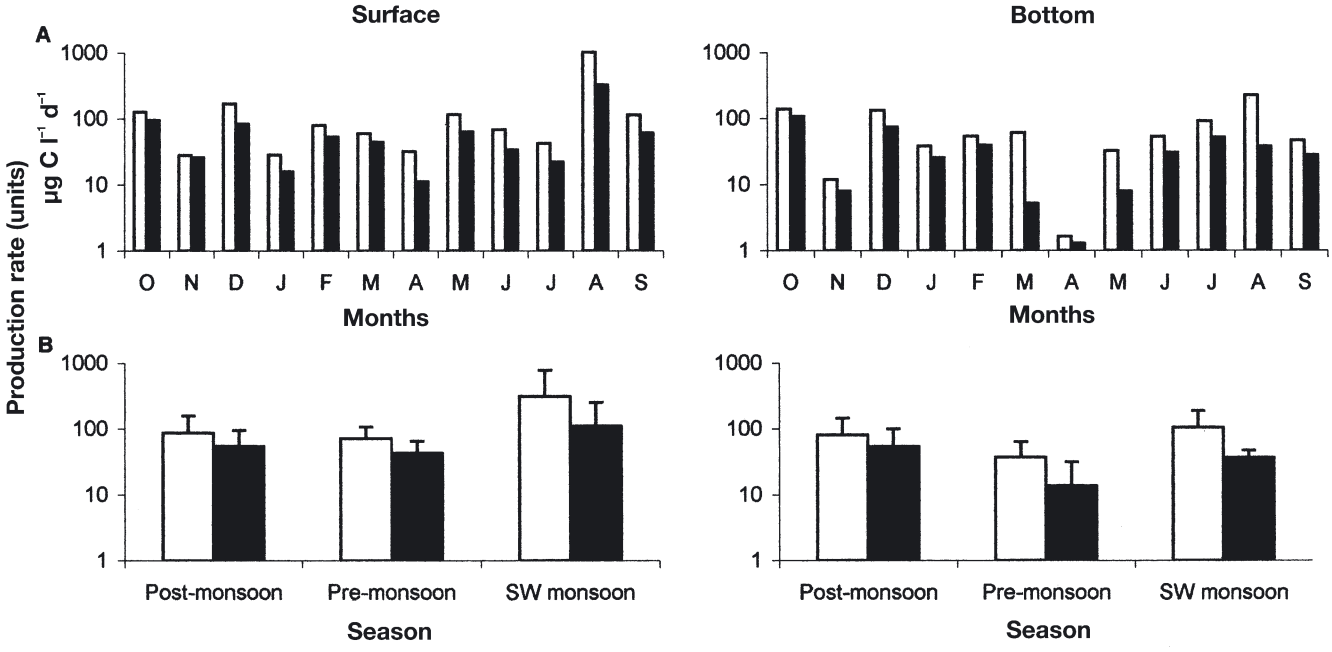

Fig. 5. (A) Monthly and (B) seasonal mean of 4 months variation along with standard deviation in total and particle-associated bacterial production (PABP) rates in surface and bottom waters. (□) Total bacteria (■) PABP

of depth integrated BCD and PP suggested that the estuarine system was autotrophic during the pre-monsoon season but shifted towards heterotrophy during the monsoon and post-monsoon seasons. A log productivity of PAB versus the log-biomass plot showed that the slope was almost parallel to the *x*-axis, suggesting top-down control (Fig. 6).

DISCUSSION

The nature of bacteria-particle associations has been the topic of sustained interest for many years (Bitton & Marshall 1980, Wotton 1994, Murrell et al. 1999, Selje & Simon 2003). Associated bacteria are usually found in low abundance (10%) compared with free-living bacteria in most open aquatic ecosystems (Kirchman 1983, Simon 1987), although in some cases they reach up to 50 to 60% of the total biomass (Pedros Alio & Brock 1983, Becquevort et al. 1998). Like many other estuarine ecosystems, the Zuari estuary exhibited a high load of suspended matter. Therefore, we separated the particle-associated bacteria by filtration through a 3 µm filter as often used in other such studies (Kirchman & Mitchell 1982, Simon 1985, Crump et al. 1998, Hollibaugh et al. 2000). The abundance of PAB in the Zuari estuary was higher than that of the free-living bacteria and at times accounted for >80% of the total abundance, which was 2- to 5-fold higher than that reported in temperate waters (Cammen & Walker 1982, Berger et al. 1996) except in the Loire estuary (France). Similarly there was a large magnitude of variation in the bacterial abundance within the estuary compared to that in temperate estuaries (Ducklow 1982, Kondratiff & Simmons 1985, Hoch et al. 1995). However, Crump et al. (1998) had observed variability in PAB every 2 h during a 28 h sampling series in the North Columbia River estuary.

In temperate estuaries, bacterial abundance seemed to be limited by one or more factors. Though Zuari estuary was influenced by the water from the adjacent areas as reflected in the variation in pH, this variable did not influence the bacterial parameters, as no relationship was observed. In spite of salinity being a variable factor in the estuary, it did not have an influence on the variation in the abundance of bacteria (Table 2). Other factors that did not affect this variation in abundance were the inorganic nature of the particles (Hoch et al. 1995, Berger et al. 1996), high number of particles (Sanudo-Wilhelmy & Taylor 1999) or input from mangroves (Bano et al. 1997). In this estuary none of the observed parameters were limiting bacterial abundance except temperature. Although temperature variation was not really strong, a negative relationship was observed. Usually a positive relationship is expected due to enhanced activity with an increase in temperature (Findlay et al. 1991b, Hoch & Kirchman 1993). However, in the Humber estuary (northeast England) (Bent & Goulder 1981) a negative relationship was due to increased suspended load brought about by influx of

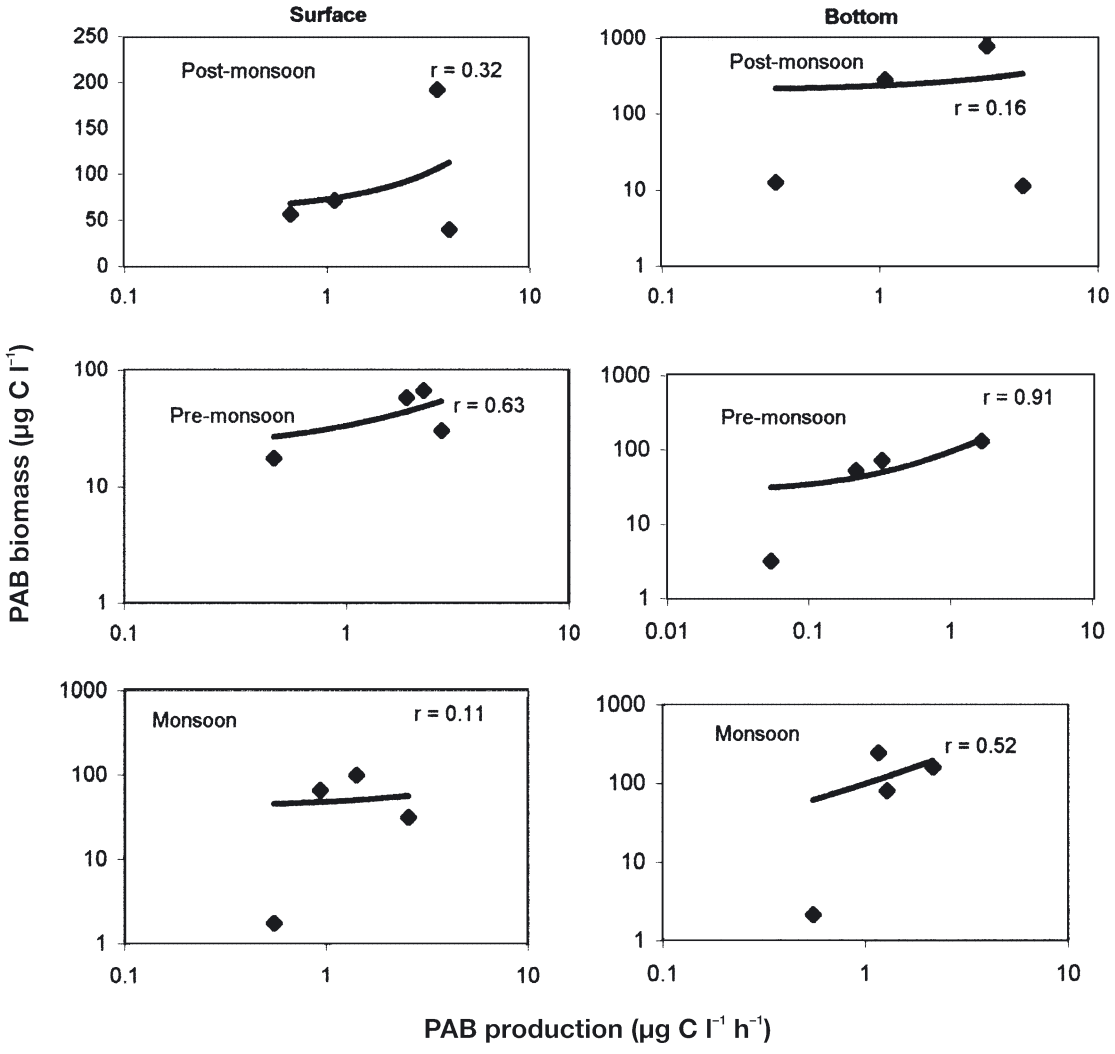

Fig. 6. Relationship between bacterial biomass and production

freshwater. In this estuary, bacterial numbers were not related to suspended load or particle numbers, suggesting that factors other than the availability of colonization sites regulate the bacterial abundance on particles (Friedrich et al. 1999). Thus the negative relationship may be due to a combination of stronger changes in other variables like precipitation, winds and salinity. In our study, the observed organic content of the bottom waters was high, which may be due to the bottom-resuspended clays bound with organic matter. Although organic carbon concentration is expected to correlate to bacterial activity, in our estuary the organic matter concentration was not related to the bacterial abundance. Murrell et al. (1999) suggested that standing crop measures of carbon was a poor predictor of bacterioplankton dynamics in San Francisco Bay (California, USA). Obviously, the nature of the organic matter rather than total organic matter is a more decisive variable (Bano et al. 1997), as the growth rate depends on the quality of the substrate (Zweifel et al. 1993). However, large variation is not unusual in aquatic regions (Iriberri et al. 1987) as the quality and quantity of the suspended particles determine the proportion of attached bacteria. Though the biochemical nature of the particles was not estimated in the present study, the C:N ratio showed a variation from 2 to 26 at the surface and from 4 to 93 at the bottom. It was inferred that during the SW monsoon, bacterial production on particles was the highest due to the input of utilizable organic matter with a low C:N ratio (mean: 4.7). This led to an increased growth rate of bacteria, as reported by Griffith et al. (1990) and Unanue et al. (1992). A high specific growth rate during the SW monsoon period supports our inference. The complex characteristics of the tropical estuary make it difficult to relate bacterial dynamics directly to its controlling factors.

The rate of bacterial production in the present study was high, and such high bacterial production was observed in the Loire estuary, in the Ivory Coast, and in a tropical mangrove tidal creek of the Indus River delta (Ducklow & Shiah 1993, Bano et al. 1997). The productivity of PAB in the present estuary was constant throughout the year as observed by Bano et al. (1997) in an Indus River delta creek (73 to 93%). This high activity, in terms of productivity throughout the year, indicates that PAB contributed significantly to high secondary bacterial production of the estuary and was not under any stress. There is recent evidence that the estuarine bacterial community undergoes physiological stress leading to reduced bacterial production (del Giorgio & Bouvier 2002), which was not observed at this station. However, during the pre-monsoon season, when the salinity values are the highest, the productivity rates are lower, especially in the bottom waters. In the temperate regions, unlike tropical estuaries, the magnitude varies from a low 7% in the Rhode River estuary (Rublee et al. 1984) to a very high 90% in the Columbia estuary (Crump et al. 1998). Seasonal changes in abundance and productivity have been reported in a variety of systems (Bent & Goulder 1981, Rublee et al. 1984, Unanue et al. 1992, Crump & Baross 1996, Sanudo-Wilhelmy & Taylor 1999) although the timing, magnitude and the likely cause of the shift may differ. Lack of large variation in our waters may be due to the absence of marked temperature differences and seasonal planktonic blooms. Although there was no pronounced variation in abundance and productivity in this station, there was an increase in abundance and productivity from pre-monsoon, to monsoon, to post-monsoon. As mentioned earlier, the increase in bacterial numbers during the SW monsoon season was due to input of easily utilizable organic matter from terrestrial regions, or the re-suspension of organic matter from sediment to the water column. Freshwater flow has been shown to be a good proxy for organic carbon input, and this could in turn limit bacterioplankton abundance (Jassby et al. 1993). As the season progressed from the pre-monsoon period, the particles could tend to become organically less labile with a concomitant increase in hydrolytic enzymatic activities during the SW and post monsoon. The temporal variation in the enzymatic profile reflected the change in nature of the particles as the season progressed. The particles get colonized in subsequent periods and may go through a high succession of sequential colonisations similar to that observed in phytoplankton detritus (Biddanda & Pomeroy 1988, Murrell et al. 1999). In line with previous reports (Crump et al. 1998, Selje & Simon 2003), PAB in the Zuari estuary is involved in the decomposition processes, as they have high hydrolytic enzymatic activities (Smith et al. 1992, Hoppe et al. 1993). In accordance with earlier findings, PAB was metabolically active as a higher biomass-specific bacterial production was observed in comparison with free-living bacteria (Kirchman & Mitchell 1982, Pedros-Alio & Brock 1983, Worm & Sondergaard 1998), which are small, inactive and in a dormant state (Jürgens & Güde 1994).

Although the bacterial production was high during the SW monsoon months, the bacterial standing stock was non-parallel to the production rate in the estuary. Such a scenario has been reported from other estuaries (Findlay et al. 1991a, Wehr et al. 1998). The population of bacteria in an ecosystem is controlled mainly by either substrate availability (bottom-up control) or by grazers (top-down). As the growth rate of PAB was higher than the unattached bacteria in both lacustrine and marine realms (Crump et al. 1998), their size was larger than the free-living bacteria (Goosen et al. 1995), hence they may be under grazing pressure. Although ingestion by grazers *per se* was not measured in the present study, the rate of bacterial production and the standing stock reflected the grazing pressure on these groups of bacteria. This is further evidenced indirectly by the decreased slope of regression of log biomass versus log production. If bacterioplankton were controlled only by substrate availability, the slope of regression would be near or equal to 1. Ducklow (1992) has theoretically defined that a slope of >0.6 would correspond to a strong effect, 0.4–0.6 to an intermediate effect, <0.4 to a weak effect, and <0.2 to no effect of substrate control. In our study the relationship was ca. 0.4, which is intermediate, indirectly suggesting both substrate control (bottom-up) and a possibility of top-down control (Fig. 6). Thus, depending on the time of collection, PAB of this estuary seems to be controlled either by bacterivores (Fuhrman & Noble 1995) or substrate availability. The role of viruses, however, has not been examined in this study but is assumed to be significant.

As PAB abundance and production formed a significant portion of the microbial biomass in the Zuari waters, the BCD would be expected to be high. The reported BGE from marine system ranged from <10 to 50% (Azam et al. 1983, del Giorgio et al. 1997). The average BGE value in our waters was 18 (±7.84%), (Ram et al. 2003). If we take the lowest and highest values for BGE, the carbon from PP (assuming 10% exudates) was not sufficient to meet the BCD, except during the pre-monsoon season. Hence it is suggested that the excess carbon requirement was met from allochthonous sources as reported by Bano et al. (1997).

Estuaries are usually net-heterotrophic as the bacterial production at certain times exceeds contemporaneous primary production (Findlay et al. 1991b) be-

cause of a high load of particulate and dissolved organic matter (Findlay et al. 1991b, Goosen et al. 1997). Heterotrophy in our study occurs when phototrophic carbon fixation is lower than BCD regardless of zooplankton requirement. The station changes temporally from an autotrophic system during the premonsoon to a heterotrophic system during the monsoon and post-monsoon seasons. Unlike turbid estuaries such as the Elbe, Westerschelde and Gironde (Germany, Netherlands and France, respectively) where heterotrophic status is achieved throughout the year (Goosen et al. 1999), in this estuary there was a shift in status. A spatial shift in status from upstream to down stream was observed in the Schelde estuary (Goosen et al. 1997). In conclusion, our study demonstrates that (1) in the tropical Zuari estuary PAB dominate bacterial processes and contributed significantly to the total secondary production, (2) the abundance of bacteria was not markedly influenced by the environmental variables, but by the availability of substrate and grazers, and (3) the bacterial carbon demand was supported by allochthonous carbon input.

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